III. CHEMICAL PROPERTIES OF PUBERULIC ACID, $C_8H_6O_6$, AND A YELLOW ACID, $C_8H_4O_6$.

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THE two acids formed from glucose by *Penicillium puberulum* Bainier and *P. aurantio-virens* Biourge were first isolated and investigated by Birkinshaw and Raistrick [1932]. Further investigations are recorded below.

Puberulic acid $C_8H_6O_6$.

Improved isolation of puberulic acid. The starting material, which was kindly supplied by Prof. Raistrick, consisted of a mixture of puberulic acid and the yellow acid $C_8H_4O_6$. This mixture had been obtained by crystallisation from methyl alcohol of the ethereal extract from the acidified nickel salts, as described by Birkinshaw and Raistrick [1932], and their method was followed in the preparation of well crystallised diacetylpuberulic acid. Dilution of the acetylation mixture with glacial acetic acid has proved to be useful in order to minimise charring. Diacetylpuberulic acid in alcoholic solution gives with $FeCl_3$ first a reddish brown and then a green colour. Brief hydrolysis with hot aqueous ammonia (a much milder method than the boiling with *N* sodium hydroxide used by Birkinshaw and Raistrick [1932]) yields analytically pure puberulic acid which may be recrystallised from water, and it is then obtained as small, practically colourless prisms. One can thus dispense with the final purification by means of high vacuum sublimation, which is attended by such losses as to give yields of only 10–15 %.

Diacetylpuberulic acid, triturated with a little water, is mixed with a small amount of concentrated ammonia solution and heated for about 10 mins. on the water-bath in a flask loosely corked in order to avoid too rapid loss of ammonia. After cooling and acidification by hydrochloric acid, a fine crystalline powder separates which should not show any needles of the unchanged diacetyl compound under the microscope. Yield 90 %. (Found: C, 48.40; H, 3.33 %. Calc.: C, 48.47; H, 3.05 %.) Recrystallisation from water must be carried out quickly; the substance is not very soluble in hot water; M.P. 300–305° (decomp.). (Found for recrystallised product: C, 48.2; H, 3.16 %.)

Decarboxylation of puberulic acid. Careful sublimation of puberulic acid at 290–310° under atmospheric pressure yields a crystalline product which is obtained in uniform, thin, glittering leaflets when the sublimation is repeated several times. This sublimed product may be recrystallised from absolute alcohol, M.P. 233–235°; reddish brown colour reaction with $FeCl_3$. (Found: C, 54.34; H, 4.37 %. $C_7H_6O_4$ requires: C, 54.53; H, 3.93 %.)

The only benzenemonocarboxylic acid agreeing in formula and M.P. with decarboxylated puberulic acid is 3:5-dihydroxybenzoic or α -resorcylic acid (M.P. 233°; no colour reaction with FeCl_3). Since the M.P. of the mixture showed a depression of 32°, these two acids are not identical.

Further evidence against puberulic acid being a benzenedicarboxylic acid. Since puberulic acid gives a distinct fluorescence when fused with resorcinol and zinc chloride (at 130–140° in an oil-bath for 10 mins.), the possibility that it might be a benzenedicarboxylic acid was again considered, although Birkinshaw and Raistrick's negative findings seemed conclusive. As a series of experiments with various aliphatic and aromatic alcohols, mono- and di-carboxylic acids showed, and as Damm and Schreiner [1882] had stated, this fluorescence does not afford a definite proof of the presence of two carboxyl groups. It was possible, however, that we were not dealing with quite the same compound as Birkinshaw and Raistrick, in view of the different conditions of hydrolysis. We therefore compared puberulic acid—prepared by the new ammonia method—with 2:4-dihydroxybenzene-1:3-dicarboxylic acid and with 4:6-dihydroxybenzene-1:3-dicarboxylic acid. We are indebted to Prof. Raistrick for specimens of these acids, the only ones deserving consideration; they are distinct from puberulic acid, which cannot therefore be a benzenedicarboxylic acid.

On the possibility of puberulic acid being a phenolic monocarboxylic acid. The alkaline hydrolysis of tetramethylpuberulic acid yields interesting information.

Hydrolysis at room temperature. A solution of tetramethylpuberulic acid in a measured amount of N NaOH was allowed to stand for 5–6 mins. at 21° and was then neutralised with N H_2SO_4 . Long, pale yellow needles crystallised out, and after recrystallisation from water had M.P. 204° and gave no colour reaction with FeCl_3 . They consisted of a trimethylpuberulic acid. (Found: C, 54.81; H, 5.25 %. $\text{C}_{11}\text{H}_{12}\text{O}_6$ requires: C, 54.98; H, 5.04 %.)

Hot hydrolysis, by boiling tetramethylpuberulic acid with a measured excess of N NaOH for 1 hour in an atmosphere of N , showed the consumption of only one equivalent of alkali on back-titration with N H_2SO_4 . The product readily crystallised from the neutralised solution and after recrystallisation from water formed pale yellow needles similar to those of the trimethyl derivative and melting at almost the same temperature, 203°. It contained only two methoxyl groups, gave a reddish brown colour reaction with FeCl_3 and a M.P. depression of 25° when mixed with the trimethyl derivative. (Found: C, 52.94; H, 4.61 %. $\text{C}_{10}\text{H}_{10}\text{O}_6$ requires: C, 53.08; H, 4.46 %.) The dimethylpuberulic acid thus prepared is not identical with that described by Birkinshaw and Raistrick [1932].

A hypothetical substituted benzenemonocarboxylic acid with a tautomeric aldehyde group could explain these observations. However it was not possible to detect an aldehyde group. Attempts to isolate and characterise a Schiff's base, hydrazone, phenylhydrazone, p -nitrophenylhydrazone or semicarbazone were unsuccessful. The Angeli-Rimini test for aldehyde groups (reaction with benzenesulph-hydroxamic acid, followed by FeCl_3) was applied to puberulic acid and its derivatives and yielded sometimes positive, sometimes negative results. Since, however, puberulic acid and its derivatives can give with FeCl_3 alone the deep coloration, which is characteristic of aldehyde groups in the Angeli-Rimini test, the results are not conclusive.

We next tried to demonstrate the enolic nature of puberulic acid, by applying Windaus's method of detecting a tautomeric aldehyde group. This method, first

applied by Windaus [1924] to colchicine, consists of oxidising the entire tautomeric aldehyde group with iodine and potassium hydroxide.

With puberulic acid, however, the results were unsatisfactory, although, according to Windaus [1923], the method is generally applicable to *o*- and *p*-hydroxy-aldehydes. Similar negative results were given by Dakin's [1909] oxidation with alkaline H_2O_2 .

The stability of puberulic acid towards neutral perhydrol was very striking. When, after heating, oxidation finally took place, a complete breakdown to CO_2 was the only detectable result. During the oxidation with chromic acid, complications arose from the formation of rather stable complex salts.

The unsaturated character of puberulic acid is shown by a distinctly positive Baeyer's test with permanganate solution as well as by a yellow coloration with tetranitromethane [Werner, 1909], and on the addition of bromine in alcoholic solution. The typical FeCl_3 reaction of puberulic acid disappears after bromination.

Catalytic hydrogenation of diacetylpuberulic acid (in stabilised glacial acetic acid with platinum oxide, according to Adams) showed a hydrogen uptake of about 6H_2 . The isolation of the oily products of hydrogenation was difficult, largely owing to the very small amounts of starting material we possessed. Free puberulic acid seemed to be much less suitable for catalytic reduction. Gradual hydrogenation with Pd and then Pt led to an uptake of hydrogen to an indeterminate extent. More certain results were obtained from the hydrogenation of tetramethylpuberulic acid in stabilised glacial acetic acid with platinum oxide. The oily end-product gave the following analytical figures: C, 58.3, 58.44; H, 8.87, 8.67; OCH_3 , 35.0, 34.92 %. $\text{C}_{12}\text{H}_{22}\text{O}_5$ requires: C, 58.55; H, 8.94; 3OCH_3 , 37.8; 4OCH_3 , 50.4 %. The figures for methoxyl deserve special attention. They represent strong evidence for a removal of an oxygen-containing group apart from a mere addition of hydrogen. The equation

must therefore include the disappearance of one methoxyl group without the loss of CH_3 . The theoretical explanation is rather difficult.

The yellow acid $\text{C}_8\text{H}_4\text{O}_6$.

Attempts to transform one acid into the other have, up to now, been unsuccessful, and there is no experimental evidence for the supposition (suggested by the formula) that the yellow acid is a quinonoid body closely related to puberulic acid¹.

The yellow acid is not decarboxylated by the usual methods, nor can it be acetylated. Experiments for the preparation of methyl or ethyl esters yielded small amounts of crystalline products, but the analytical figures of these products did not fit with any value theoretically possible for esters of the yellow acid, although they fitted well with values for esters of puberulic acid. It thus

¹ While this note was in the press Dr H. Hoyer has obtained such evidence in my laboratory. G. B.

appeared that the yellow acid used had not been free from admixed puberulic acid. Purification of the yellow acid by recrystallisation was difficult. Only very few solvents, *e.g.* nitrobenzene or ethyl acetoacetate, were of any use.

No typical aldehyde derivatives, such as Schiff's base, phenylhydrazone or semicarbazone, could be isolated. Zinc dust distillation of the yellow product in ordinary air gave a yellow sublimate of apparently unchanged material. In an atmosphere of hydrogen however an oily substance (which later solidified) was obtained in minute yield. After purification, this product had a phenolic odour and gave a positive Baeyer's test for double bonds but no distinct FeCl_3 reaction; on exposure to air it became violet.

Very interesting conditions were found on neutralising the yellow acid. The yellow colour of an aqueous solution of the acid passed into a deep pink on neutralisation, and all colour disappeared in an alkaline solution. Re-acidification produced the original yellow colour but without the pink intermediate colour. The latter appeared when this re-acidified solution was again neutralised. The play of colours was reproducible several times. A neutral pink solution, on the other hand, lost all pink colour on standing for about 30 mins. and became yellow, which colour disappeared eventually after a few hours, by which time the solution had become acid. These changes are shown diagrammatically below.

Micro-titrations gave the following figures for basicity: direct titration of an aqueous solution of the acid, 1.9. Titration of a neutralised solution (calculated for a dibasic acid) which, however, reacted acid, 2.3. Back-titration of alkaline solutions, depending upon the speed of the titration itself, figures of 2.9, 3.3, 3.5.

These phenomena closely resemble those shown by pseudo-acids. If the yellow acid is quinonoid, a quinhydrone in Willstätter's sense must be considered.

SUMMARY.

(a) *Puberulic acid.*

1. The method of isolation has been improved.
2. The substance is a monocarboxylic acid and can be decarboxylated.
3. Various methods have failed to reveal an aldehyde group, actual or potential.

(b) *Yellow acid.*

1. No aldehyde group could be demonstrated; no esters or acetyl derivatives were obtained.

2. On neutralisation the acid undergoes a curious series of colour changes, behaviour suggestive of a pseudo-acid.

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